

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
6 February 2003 (06.02.2003)

PCT

(10) International Publication Number
WO 03/010510 A2

(51) International Patent Classification⁷:

G01N

(74) Agents: REMENICK, James et al.; Intellectual Property Department, Heller Ehrman White & McAuliffe LLP, Suite 300, 101 Orchard Ridge Drive, Gaithersburg, MD 20787-1917 (US).

(21) International Application Number: PCT/US02/23348

(22) International Filing Date: 24 July 2002 (24.07.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/307,389

25 July 2001 (25.07.2001) US

(71) Applicant (for all designated States except US): ARGOSE, INC. [US/US]; 230 Second Avenue, Waltham, MA 02451 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): MESSER-SCHMIDT, Robert, G. [US/US]; 389 River Street, Waltham, MA 02453 (US). MANSFIELD, James [US/US]; 22-25 Peterborough Street, Apt. 22, Boston, MA 02215 (US). BRAND, Derek [US/US]; 11 Bentley Street, Brighton, MA 02135 (US). FREEMAN, Jenny, E. [US/US]; 405 Concord Road, Weston, MA 02493 (US). HOPMEIER, Michael, J. [US/US]; 425 E. Hollywood Blvd., Suite A, Mary Ester, FL 32580 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ADJUNCT QUANTITATIVE SYSTEM AND METHOD FOR NON-INVASIVE MEASUREMENT OF IN VIVO ANALYTES

(57) Abstract: The invention is directed to a method and apparatus for detecting and measuring the concentration of an analyte in a tissue and a patient. Particularly, the invention is directed to methods wherein a spectroscopic measurement is combined with an adjunct spectroscopic or non-spectroscopic measurement to provide a more accurate measure of the analyte. The non-spectroscopic measurements include adjunct spectral measurements, adjunct physiological measurements of the patient, the tissue or the patient from whom the tissue was obtained, or adjunct informational determinants. Preferably these methods can be used for determining the level of glucose in a patient and for enhancing a calibration system.

BEST AVAILABLE COPY

WO 03/010510 A2

ADJUNCT QUANTITATIVE SYSTEM AND METHOD FOR NON- INVASIVE MEASUREMENT OF IN VIVO ANALYTES

BACKGROUND OF THE INVENTION

1. Field of the Invention

5 The invention relates to a method and apparatus for the non-invasive measurement of *in vivo* analytes in a patient. Particularly, the invention relates to the use of a combination of one or more spectroscopic measurements combined with an adjunct spectroscopic or non-spectroscopic measurement for the measurement of analytes in a tissue of a patient.

2. Background of the Invention

10 Diabetes mellitus is a chronic life threatening disease for which there is presently no cure. It is the sixth-leading cause of death by disease in the United States, and approximately 190,000 Americans per year will die as a result of diabetes and its complications. Adopting a more global perspective, diabetes represents an enormous challenge insofar as it now afflicts an estimated 100 million people worldwide.

15 Diagnostically characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both, diabetes is now generally recognized as a group of metabolic diseases that share a common presentation and pathophysiology.

Type I diabetes (juvenile diabetes or insulin-dependent diabetes mellitus) is the most severe form of the disease comprising approximately 10% of the diabetes cases in the

20 United States. Type I diabetics must receive daily injections of insulin in order to sustain life. Type II diabetes, also known as adult onset diabetes or non-insulin-dependent diabetes mellitus, comprises the other 90% of the diabetes cases. Type II diabetes is often manageable with dietary modifications and physical exercise, but may still require treatment with insulin or other medications.

25 While diabetes represents a well-described entity, other target populations are also at risk for hyperglycemic episodes. This includes individuals with impaired glucose metabolism (i.e., impaired glucose tolerance, "IGT", insulin resistance, or impaired fasting glucose, "IFG"). These individuals have blood glucose levels that are higher

than normal but not high enough meet the diagnostic criteria typically set for diabetes. About 20 million people in the U.S. have IGT, according to the National Health and Nutritional Examination Survey III, and they are at higher risk for both diabetes (as few as 1 to as many as 10 of every 100 persons with IGT is expected to develop full blown diabetes every year) and the complications associated with chronic hyperglycemia. Similarly, a variety of other intercurrent illnesses or pathological conditions can impair glucose homeostatic mechanisms thereby predisposing hyperglycemia and its consequences.

Lacking normal glucose homeostasis, the chronic hyperglycemia associated with diabetes has now been directly linked to long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. More importantly, well-controlled research trials have documented that by testing and controlling blood glucose, people with diabetes can reduce their risk for these complications by up to 60 percent. Since assiduous management of glucose to near normal levels can prevent onset and progression of complications of diabetes, patients afflicted with either form of the disease are encouraged to monitor their blood glucose level in order to assure that the appropriate level is achieved and maintained.

Accordingly, analysis and quantification of glucose has consistently been an obvious target for helping diabetics achieve and maintain normal blood glucose levels. Some devices involve self-monitoring of glucose levels by a diabetic individual and can be performed at home, and many products for self-monitoring of blood glucose levels are available commercially. Upon recommendations by doctors and using such products, patients are typically instructed to measure blood glucose levels several times a day as a way to monitor their success in controlling blood sugar levels. Nevertheless, many diabetics do not measure their blood glucose regularly. One important reason is that the existing monitoring products may be complicated, inconvenient, costly and painful, requiring a pinprick every time a measurement is made. Furthermore, these products may also require some skill, dexterity, and discipline to obtain useful measurements.

Widespread awareness of the importance of maintaining normal glucose levels in all diabetics has prompted a wave of research and development efforts into new glucometers, which embrace less invasive techniques. As noted, most of the commercially available glucometers for home use require that blood be withdrawn.

5 Newer approaches are focusing on minimally or non-invasive technologies that would encourage diabetic self-monitoring based on ease of use and freedom from discomfort. One minimally invasive glucometer that is approved for adjunctive use relies on reverse iontophoresis, wherein the diabetic wears a proprietary patch on the skin of the arm across which a current is intermittently pulsed thereby modifying the normal epidermal permeability barrier and allowing interval sampling of interstitial fluid. A variety of
10 companies are pursuing alternative approaches to gain access to the interstitial fluid space via laser or needle microporation, chemical dissolution of the epidermal permeability barrier, or microdialysis.

Optical spectroscopy has attracted interest as well, including approaches relying on
15 either Raman, near-, mid-, or far-IR. Other innovative approaches are based on microvascular changes in the retina, acoustical impedance, NMR spectroscopy and optical hydrogels that quantify glucose levels in tear fluid.

However, despite the above potential solutions, none have been sufficiently precise, accurate, portable, affordable or easy enough to use to achieve either full FDA approval
20 or market penetration. For example, IR-based methodologies can be utilized to accurately quantitate a variety of *in vivo* parameters such as skin hydration, skin pH, skin perfusion, oxygenation, and skin temperature. However, signal modification from water and matrix components of the blood, such as hemoglobin, plus optical scatter contribute to large signal-to-noise difficulties that have resulted in variation and error in
25 measurements.

Minimally invasive approaches have experienced similar difficulty with data analysis, precision and accuracy. Specifically, reverse iontophoresis-based meters require frequent calibration, such as validation against conventional photometric or electrochemical monitors, and are reported to be uncomfortable and imprecise.

Indwelling sensors require surgical placement, tend to migrate, and demonstrate biocompatibility problems that are often secondary to a foreign-body inflammatory response, which can directly interfere with sensor function, alter glucose concentration in the interstitial fluid, or alter transit times of glucose from blood vessels through interstitial fluid. As a result, sensor readings may drop substantially shortly after
5 implantation or drift over the long term thereafter, necessitating periodic recalibration, repositioning or replacement of the unit.

Thus, there is a clear need for a simple and accurate method and device for non-invasively measuring *in vivo* analyte concentrations, such as glucose. Accordingly,
10 described herein is a simple and accurate method and device for non-invasively measuring blood analyte concentration, particularly in the context of blood glucose monitoring in a patient.

SUMMARY OF THE INVENTION

In relation to the above, the unmet need of diabetics (including pre-diabetics) is
15 addressed by the instant invention which combines spectral and non-spectral measurements and techniques to measure biological analytes, such as glucose.

Accordingly, in a preferred embodiment, the invention provides a method and apparatus to supplement ultraviolet-visible fluorescence measurements with one or more additional or adjunct measurements for quantitating an analyte, such as glucose.

20 A preferred embodiment of this invention is directed to a method for the *in vivo* measurement of at least one biological analyte through tissue exposure to radiation, followed by spectroscopic analysis, preferably selectively evaluating ultraviolet and/or visible light fluorescence, in combination with at least one adjunctive optical measurement selected from the group comprising infrared (IR) which includes near
25 infrared (NIR), mid infrared (MIR) and far infrared (FIR), visible light absorbance, Raman, microwave and/or combinations thereof. Alternatively, the spectroscopic measurement is combined with at least one adjunct physiological parameter measurement and/or at least one adjunct informational parameter measurement. These

types of adjunct measurements can also be utilized to accomplish and/or enhance the calibration of an analyte level quantitation device.

5 An advantage of the above embodiment is that robustness, sensitivity, specificity, and/or accuracy are added to the non-invasive measurement of the analyte (e.g. glucose), thereby reducing the error of the measurement. At the same time the technology has a clearly viable miniaturization and cost reduction strategy.

10 Another advantage of the invention is that the signals detected from water absorption, blood components such as hemoglobin absorption, and optical scatter, which are potential sources of variation and error in measurements, are accounted for, rather than factoring their variance into measurements as error.

15 In a preferred embodiment, the invention provides a method of determining a level of at least one analyte in a tissue comprising: exposing the tissue with an excitation radiation from an excitation source; detecting a spectral emission or absorption from the excited or illuminated tissue; determining an adjunct parameter selected from the group comprising: at least one adjunct spectral emission; at least one adjunct physiological determinant; at least one adjunct informational determinant; and a combination thereof; combining the spectral emission detected with the adjunct parameter determined, and determining the level of said at least one analyte in the tissue.

20 Preferably, the excitation source provides electromagnetic radiation such as fluorescence, visible light, ultraviolet radiation, IR radiation such as FIR, MIR or NIR, microwaves and combinations thereof. The source preferably comprises exposing said tissue and exciting a target within the tissue.

Preferably, the target is a structural, cellular, matrix, or molecular species in a patient.

25 Preferably, the structural, cellular, matrix, or molecular species is selected from the group comprising pepsin- or collagenase-digestible collagen cross links, non-pepsin digestible collagen cross links, tryptophan, elastin, elastin cross-links, keratin, serum proteins, Glu-T proteins, NADH, NADPH, flavoproteins (e.g. FAD), melanin precursors, porphyrins (e.g. including hemoglobin, glycosylated hemoglobin A1c, or red

blood cells), cytochromes, vitamin B complexes, carotenoid, salicylate (aspirin), lactate, pyruvate, ketones (e.g. acetoacetate and beta-hydroxybutyrate), free fatty acids, succinate, fumarate, dihydroxyacetone phosphate (DHAP), 3-phosphoglycerate, acetyl CoA, succinyl CoA, alpha-ketoglutarate, malate, citrate, isocitrate, bicarbonate, insulin, triglyceride, cholesterol, phosphorus, calcium, blood urea, electrolytes, bilirubin, creatinine, albumin, lactate dehydrogenase (LDH), and combinations thereof.

Preferably, the level of the at least one analyte is a relative or an absolute amount.

Preferably, at least one analyte is selected from the group comprising glucose, NADH, NADPH, FAD, tryptophan, collagen, elastin, salicylate (aspirin), lactate, pyruvate, ketones (acetoacetate and beta-hydroxybutyrate), free fatty acids, succinate, fumarate, DHAP, 3-phosphoglycerate, acetyl CoA, succinyl CoA, alpha-ketoglutarate, malate, citrate, isocitrate, bicarbonate, insulin, hemoglobin, glycosylated hemoglobin A1c, triglycerides, cholesterol, phosphorus, calcium, blood urea, electrolytes, bilirubin, creatinine, total protein, albumin, LDH, blood gases, cholesterol, alcohol, medications, pharmaceuticals, narcotics (e.g. cocaine), and/or poisons (e.g. cyanide).

Preferably, the tissue is selected from the group comprising human tissue, animal tissue, forensic tissue, skin, soft tissue of the mouth, ear lobe tissue, internal body tissue, eye tissue, tissue in or around an eye, internal organ tissue, a whole organism and combinations thereof.

Preferably, the excitation radiation is at a wavelength between about 295 nm to about 1100 nm. More preferably, the excitation radiation is at a wavelength between about 320 nm to about 700 nm. Even more preferably, the excitation radiation is at a wavelength between about 320 nm to about 510 nm.

Preferably, the excitation source is a visible light source, a laser source, a microwave source, a discharge light source, an incandescent light source, a light emitting diode (LED), or a fluorescent light source.

Preferably, the spectral emission or absorption is IR, Ramen, ultraviolet, visible, or fluorescence radiation.

Preferably, at least one spectral emission is detected at a wavelength between about 295 nm to about 700 nm.

Preferably, the spectral emission comprises measuring one or more spectral characteristics of the excited target selected from the group comprising fluorescence life-time, wavelength, intensity including peak heights and peak areas, relative peak ratios, spectral shapes, peak shifts, band narrowing, spectral kinetics, band broadening, scattering, polarization and combinations thereof.

Preferably, the spectral emission is performed substantially simultaneously with determination of said adjunct parameter.

10 Preferably, the detection of the spectral emission is performed substantially sequentially with determination and/or data entry of the adjunct parameter.

Preferably, the selected target and detection of the spectral emission are performed at the same or different locations of said tissue.

15 Preferably, at least one adjunct spectral emission or absorption comprises infrared including NIR, MIR, and FIR, visible absorbance, Raman or microwave.

Preferably, at least one adjunct physiological determinant is determined by a passive or active procedure.

20 Preferably, at least one physiological determinant comprises a combination of one or more local or systemic determinants selected from a group comprising: oxygen saturation (arterial and/or venous), temperature, blood volume, one or more blood chemical determinants, blood volume change, blood pressure, cardiac output, blood flow, pulsatile effects, pH, skin perfusion, hydration, vasodilation, nitric oxide (NO), metabolic index, or respiratory function parameters (e.g., oxygen partial pressure and carbon dioxide partial pressure), as well as other tissue optical properties, such as
25 contact pressure/optical interface, tissue color, tissue homogeneity, skin roughness, skin stretch, skin tone, ultraviolet effects, ultraviolet dosage, and the like, and combinations thereof.

Preferably, at least one informational determinant is a combination of one or more determinants selected from the group comprising date, time of day, period of time since last meal, content of last meal, insulin dosing information (e.g. period of time since last insulin injection, dosage of last insulin injection, insulin pump data), age of the patient, skin age of the patient, skin AGE determination, skin color, gender, menstrual history, weight, percent body fat, exercise/activity level, pulse rate, type of diabetes, duration of diabetes, type and dosage of non-insulin medications, medical complications secondary to diabetes, pertinent additional medical and family history, aspirin usage, tobacco and/or alcohol usage, blood/serum osmolality and analyte determination category. In a preferred embodiment the informational determinant will also include pulse oximetry data reflecting relative or absolute levels of oxy:deoxyhemoglobin.

Preferably, combining of the spectral emission with the adjunct parameter comprises aliasing and folding. Preferably, the multivariate technique is selected from the group comprising partial least squares (PLS) (e.g. linear or non-linear), principal components regression (PCR), ridge regression (RR), linear discriminant analysis (LDA), linear regression (LR), multiple linear regression (MLR), locally weighted regression (LWR), stepwise linear regression, neural net analysis (NN), and combinations thereof.

Preferably, calibrating the spectral information against one or more other spectral emissions from said target using one or more multivariate techniques.

Preferably, the instant method further comprises detecting a vibrational-spectroscopic emission from the excited tissue. Preferably, the vibrational-spectroscopic emission is collected at a frequency common to the detected spectral emission.

Preferably, the instant method further comprises adjusting a level of analyte determined by incorporating spectral information with a spatial dimension, a temporal dimension, or a combination thereof.

In another preferred embodiment, the instant invention provides an apparatus for determining a level of at least one analyte in a tissue comprising: a light source for exciting a target in the tissue with excitation radiation; a detector for detecting at least

one spectral emission from the excited target; a determining means for determining, recording or collecting an adjunct parameter; and a means for combining said spectral emission detected with said adjunct parameter to obtain the level of the at least one analyte in the tissue.

- 5 Preferably, the instant apparatus further comprises a display element, wherein said display element displays the level of the at least one analyte in the tissue.

Preferably, the instant apparatus further comprises a transmitting means for transmitting data pertaining to the detected spectral emission, the adjunct parameter, the level of the at least one analyte, or any combination thereof.

- 10 Preferably, the excitation radiation is at a wavelength selected in a range from about 295 nm to about 1100 nm.

Preferably, the detection means detects emission radiation at one or more wavelengths in the range from about 295 nm to 700 nm.

- 15 Preferably, the instant apparatus further comprises an audio generator, wherein said audio generator generates one or more sounds when said level at least one analyte is determined.

Preferably, the means for combining is a processor. Preferably, the processor comprises an algorithm to combine said spectral emission and adjunct parameter.

Preferably, the instant apparatus further comprises a memory.

- 20 Additional objects, features and advantages of the invention will be set forth in the description which follows, and in part, will be obvious from the description, or may be learned by practice of the invention. The objects, features and advantages of the invention may be realized and obtained by means of the instrumentalities and combination particularly pointed out in the appended claims.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Optical information from tissue can be used to indicate the physiological state of the tissue and often the patient from which the tissue was obtained. For example, IR such as near-IR methods can be utilized to quantitate a number of useful *in vivo* parameters such as skin hydration, skin pH, skin perfusion, and oxygenation, as well as skin temperature. Many of these parameters can be quantitated using commercially available instrumentation. However, signals seen from water absorption, or blood signals such as hemoglobin absorption and/or scattering are potential sources of variation and error in measurements.

10 The phenomenon of endogenous skin fluorescence, as well as endogenous fluorescence of other biological tissues, is well known in the field. The fluorophores responsible for skin autofluorescence in the ultraviolet and blue regions of the spectrum include metabolic components and intermediates, plus proteins and collagen. This includes tryptophan, which fluoresces in the 295-350 nanometer (nm) region, keratin, which
15 fluoresces in the 295-340 nm region, nicotinamide adenine dinucleotide ("NADH"), which fluoresces in the 460 nm region, flavin adenine dinucleotide ("FAD"), which fluoresces in the 525 nm region, and fluorophores associated with collagen cross-links, which fluoresce in a broad region from 420 to 490 nm. (J. Invest. Dermatol. 111:776-780, 1998, and references therein). The collagen cross-link fluorophores are thought to
20 arise through a number of possible chemical transformations, including the Maillard reaction, into stable entities known as advanced glycosylation end products (AGE's). These AGE's form at a higher rate in people with diabetes presumably because of chronic exposure to elevated of tissue glucose levels.

Beyond the modulations induced by prandial ingestion and insulin dosing, contributing
25 factors to the dynamic glucose fluctuations seen in diabetics may result from environmental changes in the tissues immediately surrounding capillaries reflecting relative hydration, osmolality, and local metabolic phenomena such as reversible glucose binding to collagen. These types of environmental changes can contribute to tertiary structural changes that affect fluorescence spectra and whose modulations seem

to be directly associated with fluctuation in blood glucose levels plus incorporated contributions from other glucose dependant fluorophores.

Various spectroscopic methodologies have been widely documented for the assessment of tumors and other pathological conditions. However, spectral and non-spectral
5 measurements and techniques have not been combined heretofore to measure a specific analyte using both analyte-specific and non-analyte-specific methodologies.

The present invention provides methods and devices for *in vivo* quantitation and trend analysis of biological analyte concentrations in tissue. The present invention is directed to a methodology and apparatus that is able to combine ultraviolet and visible light
10 spectral analyses with one or more other spectroscopic techniques and/or one or more physiological measurements and/or informational parameters for the purpose of improving analyte level determination.

In general, the invention is directed to measuring a level of at least one analyte in a patient, which is usually a mammal and preferably a human, that is accomplished by
15 exciting a target in a tissue by radiation and performing at least one spectroscopic visible or ultraviolet light fluorescence emission measurement.

Analytes that may be detected by methods of the instant invention, include, but are not limited to, glucose, NADH, NADPH, FAD, tryptophan, collagen, elastin, salicylate (i.e. aspirin), lactate, pyruvate, ketones (e.g. acetoacetate and beta-hydroxybutyrate), free
20 fatty acids, succinate, fumarate, dihydroxyacetone phosphate (e.g. DHAP), 3-phosphoglycerate, acetyl CoA, succinyl CoA, alpha-ketoglutarate, malate, citrate, isocitrate, bicarbonate, insulin, hemoglobin, glycosylated hemoglobin A1c, triglycerides, cholesterol, phosphorus, calcium, blood urea, electrolytes, bilirubin, creatinine, total protein, albumin, lactate dehydrogenase (LDH), blood gases,
25 cholesterol, alcohol, medications, narcotics, and/or poisons (e.g. cyanide).

Suitable targets are those that reflect alterations within the environment of matrix components of the skin or other tissue and are sensitive to, or correlate with, analyte levels when exposed to ultraviolet or visible light radiant energy. In a preferred

embodiment, ratiometric analysis of multiple targets (e.g. NADH/FAD or oxy/deoxyhemoglobin) or integrating adjunct data in a ratiometric fashion which contribute information about local environmental perturbations that similarly are sensitive to, or correlate with, analyte levels when exposed to ultraviolet or visible light radiant energy.

In a preferred embodiment, a target excited by ultraviolet or visible light radiation may contribute to characteristic diffuse reflectance emissions. The measurement of photons lost through elastic scatter, absorption or both can provide an additional indirect measure of glucose through casual perturbations. In this way, a target can be used as a bioamplifier or a bioreporter, facilitating the analysis and quantitation of non-fluorescing or emitting analytes. For example, changes in oxygen saturation (sO_2), total hemoglobin, microcirculation (e.g. vasodilation and/or vasoconstriction), hydration, temperature, interstitial intravascular or intracellular particulate size, and/or density contribute to distinct changes in diffuse reflectance spectra. Since these spectral shapes are dependant upon scatter and absorption properties of the tissue being examined, *in vivo* metabolic and physiologic perturbations indicated by changes in the spectral shapes of the diffuse reflectance emissions add valuable information to analyte quantification.

In a preferred embodiment, spectral information obtained by excitation with ultraviolet or visible radiation can be combined with pulse oximetry data to yield specific analyte and/or physiological information.

Alternately, a target may be a quencher and yield a characteristic signal which can be correlated with an analyte level. Fluorescence quenching is a process which decreases the intensity of the fluorescence emission. The accessibility of groups on a protein molecule can be measured by use of quenchers to perturb fluorophores and decrease fluorescence. Quenching may occur by several mechanisms which include collisional or dynamic quenching, static quenching, quenching by energy transfer, or charge transfer reactions. Data provided by pulse oximetry may be a useful adjunct for correlating analyte levels with quenching signal profiles. This is especially important when considering potential changes in oxygen saturation that may occur at a global

systemic level, at a level restricted to a given extremity, or as a manifestation of a difference between central and peripheral oxygen saturation. These types of changes can occur in the face of thermal gradients (e.g. environmental or perfusion dependent), physiological accommodations (e.g. exercise or stress induced) or between temporally
5 discrete measurements.

In a preferred embodiment, targets for excitation include structural, cellular, matrix or molecular tissue components or combination of components of the patient. Preferably, the targets include, but are not limited to, the skin or any of its components or appendages, such as pepsin- or collagenase-digestible collagen cross links, non-pepsin
10 digestible collagen cross links, tryptophan, elastin, elastin cross-links, keratin, serum proteins, Glu-T proteins, NADH, NADPH, flavoproteins (e.g. FAD), melanin precursors, porphyrins (including hemoglobin, glycosylated hemoglobin A1c, or red blood cells), cytochromes, some vitamin B complexes, and other chromophores, such as carotenoids, and combinations thereof. Other preferred targets may include salicylate
15 (aspirin), lactate, pyruvate, ketones (acetoacetate and beta-hydroxybutyrate), free fatty acids, succinate, fumarate, dihydroxyacetone phosphate (DHAP), 3-phosphoglycerate, acetyl CoA, succinyl CoA, alpha-ketoglutarate, malate, citrate, isocitrate, bicarbonate, insulin, triglycerides, cholesterol, phosphorus, calcium, blood urea, electrolytes, bilirubin, creatinine, total protein, albumin, lactate dehydrogenase (LDH), and
20 combinations thereof that are related to, sensitive to, or co-vary with analyte concentration.

The contributions of diffuse reflectance (DR) to ultraviolet-visible fluorescence measurements creates a novel synergy that expands *in vivo* analyte detection options. Spectrally distinct from fluorescence, DR herein refers to light that is elastically
25 scattered from tissue without any change in wavelength. The diffuse nature arises from scatter that invariably occurs in a medium that has an inhomogeneous distribution of refractive index. Examples of such include variations in the structure, size and density of intravascular, intracellular and/or interstitial components giving rise to spatial and/or temporal variations in refractive index. In this regard, the character of the scatter and the
30 information it contains obviously depends, among other things, on whether the scatter

arises from temporal or spatial variations in refractive index. For example, optical coherence tomography utilizes back-scatter from spatial structures for imaging purposes and laser doppler velocimetry utilizes the temporal nature of the scattered light to ascertain velocity information. In another example taken from the field of flow cell cytometry, spatial scatter profiles provide cell size information. In biological tissues, the large number and varying distributions of different types of scatter centers as well as variations in the refractive index of the surrounding medium will complicate the precise nature of scatter in such media. The wavelength used, optical polarization, angle of illumination, and the way in which the diffuse reflected light is detected can have a significant impact on the nature of the spectrum.

A convenient way to describe a diffuse reflected spectrum (when more than one wavelength of light is used to interrogate the tissue) is to take the logarithm of the ratio the signal to the incident energy spectrum. In transmission absorption spectroscopy, this is routinely done to provide estimates of concentrations of absorbers in the medium when the Beer-Lambert law is known to apply. Although the Beer-Lambert law does not apply in tissue reflectance studies, it is a useful transformation. The resulting "absorbance" spectrum contains features that may be useful in identifying constituent components although quantification must also be addressed because: (1) scatter introduces a loss mechanism whereby the absorbance spectrum exhibits 'apparent' absorption characteristics even in the absence of truly absorbing chromophores and this loss mechanism is wavelength dependent, (2) scatter gives rise to a distribution of path length samplings (also wavelength dependent) whereby concentrations of true chromophores present in the medium can not be simply extracted from the absorbance spectrum and knowledge of the chromophores extinction coefficient, (3) the absorbance spectrum can be very dependent on the configuration used to measure the DR spectrum, and (4) temporal variations in the scatter environment will have nonlinear effects on the distribution of path lengths with consequent further complications in chromophore concentration extraction and apparent absorption due to scatter. Despite these complications, in fact because of them, useful information is contained in such a spectrum.

In the preferred embodiment, the DR spectrum is an optical measurement of the same tissue region from which auto-fluorescence arises and hence enhances the accuracy, specificity and sensitivity of analyte measurements, including glucose.

With respect to the practical (such as engineering) implementation of DR in a fluorescence-based product, the instrument already has the necessary components to do such a measurement. The light sources and spectral filtering are essentially the same. Instrument performance requirements may differ between DR and fluorescence measurements in the following areas: (1) resolution of the spectrometer (depending on use of DR spectrum); (2) dynamic range of detector; (3) reference measurement; and (4) interface probe design.

I. Fluorescence Spectrum Correction

Correction for absorption and scatter

One use of diffuse reflectance emission is to correct the fluorescence spectra for the perturbations arising from absorption of chromophores and the loss due to scatter (scatter losses have spectral signatures that are dependent on skin optical properties as well as probe geometry). The affect on the fluorescence spectra arising from temporal and spatial variations in chromophores and their densities as well as temporal and spatial variations in scatter could be eliminated or minimized.

A zero-order approach is to assume that the difference in source location and fluorophore location is not a factor. The source light and the fluorescence light both traverse the same region of tissue and are therefore similarly affected. The light source at the surface of the specimen for a DR measurement can be viewed as a known fluorophore (via a reference measurement). Therefore, the resulting DR spectrum contains the perturbation effects of light scatter or absorption of skin and chromophore absorption. Correcting the fluorescence spectra for these scatter and absorption perturbations amounts simply to inverting the absorbance spectrum, taking the square root, and multiplying by the fluorescence. More exact corrections have been described in the publication "Intrinsic fluorescence spectroscopy in turbid media: disentangling

effects of scattering and absorption" by M. G. Muller et. al., Applied Optics Vol. 40 # 25 pp 4633-4646 (2001).

Correction for biological or probe interface noise. One source of variations in fluorescent spectra are probe interface instabilities (motion-related artifacts). Additional sources may arise from temporal changes in the tissue biology. DR can provide a way to track such temporal changes and correct fluorescent spectra for these changes.

One source of variations in fluorescent spectra are probe interface instabilities (motion-related artifacts). Additional sources may arise from temporal changes in the tissue biology. DR can provide a way to track such temporal changes and correct fluorescent spectra for these changes.

II. Skin Classification

In past clinical studies, model stability is compromised after introduction of specific individuals to the model. There are no obvious differences in the fluorescence spectra of such individuals when compared with others. DR will provide additional information directly relating to the optical properties of the individuals' skin (the same properties that affect fluorescence) that may provide a more quantifiable means for distinguishing between individuals. This may, for example, provide for one type of model vs. another. This then would lead to improved model stability. Possible means of classifying DR spectra include the correlation of selected parts or all of the DR spectrum with various measured quantities such as fluorescence intensity across individuals or sites, sites within an individual, individuals alone, a measure of skin color, temperature, pressure, perfusion, arm position, etc.

III. Explicit glucose information to improve specificity and selectivity.

The DR spectrum itself can contain signatures for glucose concentration. For example, some studies have shown that scatter changes with glucose concentration. Selectivity and specificity are at issue here since many other things affect scatter. By combining DR with fluorescence, selectivity and specificity can be improved. There may be other

mechanisms through which glucose concentration may affect DR, for example, particles whose scatter or absorptive properties are dependent on local glucose concentration.

IV. Physiology Information

Parameters measuring the oxygenation state of blood and blood volume can be determined from a DR spectrum. The utility of this information for glucose prediction arises from the fact that glucose is delivered to tissues via blood. Furthermore, DR is affected by changes in scatter and absorption. These, in turn, can be caused by refractive index variations (caused by analyte concentration changes, hydration changes, temperature changes, etc.), particle (cells and their components) size changes, and chromophore concentration changes (e.g. absorption) that correlate to physiological state of the tissue. In a preferred embodiment these parameters can be integrated into the dataset to yield information relating to dynamic blood flow, metabolic activity, exercise tolerance, and other physiological conditions that can be mapped by analyte flux. DR spectral information derived either statically or in serial sets can facilitate blood flow corrections that correct for local vs. systemic processes. The use of perfusion information as an adjunct may provide useful information as to the effects of oxy- and deoxyhemoglobin on the spectra collected. Additionally, perfusion information can provide information as to the delivery and uptake of nutrients and the removal of metabolic byproducts. These parameters can be useful in determining analyte levels.

The application of algorithmic calculations and/or modeling to fluorescence spectra collected from such tissues exposure to radiation will determine the concentration of said analyte (e.g. see Thomas EV. Adaptable multivariate calibration models for spectral applications *Anal Chem.* 72(13):2821-7 (2000); Heise HM Non-invasive monitoring of metabolites using near infrared spectroscopy: state of the art. *Horm Metab Res.* (10):527-34 (1996); Martens, H. and Naes, T. Multivariate Calibration, John Wiley and Sons, Chichester (1993)).

A fluorescence spectra of a given patient is obtained by choosing at least one excitation wavelength from the UV-visible region, exposing between about 295 nm and 1100 nm,

preferably between 320 nm and 700 nm and most preferably between 320 nm and 510 nm, and collecting the emission spectra at one or more wavelengths, most preferably in the region from 320 nm to 650 nm. In alternative embodiments, any range or number of distinct or overlapping wavelength ranges can be selected for excitation or collection depending on the desired target within the tissue and the selected spectral emission to be analyzed. The step for measuring fluorescence comprises analyzing one or more parameters relating to fluorescence life-time, wavelength, intensity, peak location, relative peak ratio, spectral shape, peak width, peak shift, band narrowing, fluorescence kinetics, band broadening, scattering, phase, time, polarization, and the like, or combinations thereof.

Without intent of being bound by theory, changes in analyte concentration, which are expected to be associated with reversible changes in observable fluorescence of a target can be detected and analyzed. These changes may be due, in part, to direct and/or indirect effects of an analyte or other molecules on the environment. Analyte molecules in the environment may be covalently or non-covalently coupled to a target, may affect a target without binding, or may simply exist unbound in the immediate vicinity of a target. Alternatively, the fluorescence of the target may remain constant and the effects of the environment or the intervening tissue may influence the signal recorded, or both the fluorescence of the target and intervening effects may change and produce data that can be seen to co-vary with analyte levels.

In the context of glucose monitoring, chronic hyperglycemia associated with diabetes or impaired glucose tolerance, IGT, progressively affects collagen and other skin components, such as pepsin-digestible collagen cross links (Monnier et al., Diabetes 37:867-872, 1988). Accordingly, corresponding spectral emissions are expected to reflect not only dynamically shifting tissue glucose levels, but more chronic glycosylation changes as well. Some of these glycosylation-induced changes are reversible, facilitating instantaneous and longer-term analysis opportunities. Although glucose itself does not fluoresce to any significant degree, changes in glucose concentration, which are associated with reversible changes in the observable fluorescence of a target, such as collagen, can be detected. These changes may be due,

in part, to the direct and/or indirect effects of the presence of glucose or other molecules on the environment of target molecules and structures.

In a preferred embodiment, spectroscopic measurements are combined with at least one adjunctive measurement. An adjunct measurement or parameter is any information or data that aids and/or improves an analyte quantitation technique, such as ultraviolet-visual fluorescence.

Preferably, ultraviolet-visible light spectral data is combined with NIR absorption data. The NIR data allows measurement of water concentration, and contains specific information about pH, hemoglobin, organic composition, and tissue temperature.

In a preferred embodiment, a NIR spectrum of hemoglobin is obtained. Hemoglobin is a protein found within erythrocytes and has strong absorbance bands in the visible and NIR spectrum. Oxy-hemoglobin absorbs at 410 nm, 540 nm, and 580 nm and by taking absorbance measurements at these wavelengths it is possible to deduce total blood volume and oxygenation state. Additional physiological information can be obtained by pairing O₂ saturation information with fluorescent spectra specifically including, but not limited to NADH (or NADPH) or ratiometric NADH/FAD data.

By weighting the measurements at either 410 and 436 nm or 540, 560, and 580 nm the instant invention provides a method to weight spectral features according to the depth at which they originate, thus providing a depth discriminating feature. Depth delineation (depth of spectral examination) is effected by spectroscopic identification of intravascular hemoglobin thereby also providing a determination of the amount of hemoglobin in the tissue being scanned. The detection of hemoglobin and hence determination of the depth from which the signal has been acquired can be used to trigger the collection of spectral data. Furthermore, by incorporating a pulsatile (i.e. relating to pulse or heartbeat) measurement or trending measurement along with each of the aforementioned wavelengths, the total blood flow can be calculated. The oxygenation state of the tissue can be further derived from the rates of production of bio-molecules such as NADH, NADPH, FAD, and other analytes in the metabolic chain, which may be calculated. Therefore, it can be appreciated that depth

discrimination refers to the relative depth normal to the surface of the skin that the preferred analyte signal emanates from. The delineation of depth can provide specific information to an algorithm designed for the calculation of analyte concentration. For example, if the concentration of a particular analyte in skin is related to blood flow, the concentration may follow a depth-dependent gradient from the source of the analyte (e.g. the capillary bed which feeds nutrients to the upper dermis and epidermis of skin).

The concentration of the analyte at a particular distance from the source can be determined mathematically and an algorithm, with the input of a depth parameter, can adjust the calculated analyte quantity to express that value relative to the depth at which it was sampled.

Furthermore, by incorporating a pulsatile (i.e. relating to pulse or heartbeat) measurement or trend in measurement along with each of the aforementioned wavelengths, the total blood flow can be calculated. The oxygenation state of the tissue can be further derived from the rates of production of bio-molecules such as NADH, NADPH, FAD, and other analytes in the metabolic chain, which may be calculated.

In a preferred embodiment, NIR and/or Raman generated water information may be correlated with physiological tissue perfusion. In addition, the NIR region contains spectral information from glucose absorption (see Heise ref.). Accordingly, adjunct data obtained by NIR may be used to calibrate and improve the precision of ultraviolet-visible light fluorescence data. There are at least two major ways in which adjunct NIR spectroscopic measurements could improve fluorescence calibrations: 1) by using the water absorbance band in the NIR as a means by which to quantitate the amount of water in the tissue (tissue hydration state) and including this information in the fluorescence calibration; and 2) by including spectral regions that contain information about glucose absorbances in tissue directly in the fluorescence calibration.

In a preferred embodiment of the invention, the ultraviolet-visible light spectroscopic measurement is combined with at least one adjunct physiological parameter measurement. The physiological parameter can be measured via passive measurement techniques or alternatively, actively induced measurement techniques. The

physiological parameter may be oxygenation, oxygen saturation, temperature, blood volume, blood volume change, blood pressure, blood flow, pulsatile effects, pH, skin perfusion, hydration, vasodilation, nitric oxide (NO), carbon dioxide (CO₂), as well as other tissue optical properties, such as contact pressure/optical interface, tissue color, tissue homogeneity, skin roughness, skin stretch, skin tone, ultraviolet effects, ultraviolet dosage, and the like.

In another embodiment of the invention, the ultraviolet-visible light spectroscopic measurement is combined with at least one adjunct informational parameter measurement. The informational parameter is one or more parameters selected from a group comprising date, time of day, period of time since last meal, content of last meal, drug dosing information such as period of time since last drug administration, dosage of last administration, insulin pump data, age of the patient, skin age of the patient which is the apparent age as determined by build-up of fluorescent AGEs in the skin, skin color, gender, menstrual history, weight, percent body fat, exercise and activity level, pulse rate, type of diabetes, duration of diabetes, type and dosage of non-insulin medications, medical complications secondary to diabetes, pertinent additional medical and family history, aspirin usage, tobacco and/or alcohol usage, and analyte determination category.

In a preferred embodiment, the ultraviolet-visible light spectroscopic measurements are combined with any combination of at least one adjunct physiological parameter measurement, at least one adjunct informational parameter measurement, and at least one adjunct spectroscopic measurement selected from the group comprising infrared, near infrared, ultraviolet and visible light absorbance.

The ultraviolet-visible light spectroscopic measurements can be performed substantially simultaneously or sequentially with the adjunct measurements. Further, the ultraviolet-visible light spectroscopic measurements and one or more adjunct measurements may be performed at the same or different physical location on the patient.

There are four general categories of methods by which adjunct information can be added to fluorescence spectroscopic measurements to improve the ability to quantitate

the level of an analyte. The adjunctive data provides characteristics of the individual by introducing demographic, physiologic, medical, and other relevant information by the following means:

- 5 1) For adjunct spectroscopic methods (*e.g.* diffuse reflectance or Raman spectroscopies) combined with fluorescence, the simplest method is simple concatenation of the spectra prior to multivariate analysis. The scaling and weighting of the relative importance of the two spectra will be handled by the multivariate methodology.
- 10 2) For adjunct spectroscopic methods (*e.g.* diffuse reflectance or Raman spectroscopies) combined with fluorescence, one can calculate a form of reduced basis set for the adjunct spectra prior to concatenation with the fluorescence spectra. Basis set reduction means reducing a set of data down to some more manageable reduced set of variables through the application of some form of algorithm. Basis set reduction, as a general technique is very common in science (John Stuart Mill, A System of Logic: Ratiocinative and Inductive, variorum edition in Collected Works, vols. 7-8, J.M. Robson, ed. (Toronto, 1973), as it involves reducing a large number of variables down to a smaller number of variables that contain all of the information of the larger number of variables. For spectroscopic applications, examples include: spectral fitting; PCA; oxygen saturation calculations; peak area measurements; peak location measurement; peak shape measurements; quantification of some analyte or value from the spectra through univariate or multivariate means; wavelet transforms; *etc.* This reduced basis set is then concatenated with the fluorescence spectrum and multivariate analysis performed. Adjunct measurements that already comprise a reduced basis set (*viz.*, they are the summary of some other measurement (*e.g.* pulse rate, blood pressure, blood analyte values measured from a blood sample, height, weight, age, body fat percentage, air temperature, *etc.*) that was taken either simultaneously or not simultaneously with the fluorescence measurement) can simply be concatenated with the fluorescence spectrum prior to multivariate analysis.
- 25 3) Adjunct measurements, either by themselves or following some form of basis set reduction, can be used as a means of categorizing multivariate calibration models and

choosing which one or ones to apply. Multivariate calibration models are only applicable to the set of variables and conditions on which they were modeled. The adjunct measurement can be used as the criteria by which the selection of the most appropriate multivariate calibration model is done.

5 4) The adjunct measurement or some reduced basis set form of the adjunct measurement can be used as a direct input into the algorithm or multivariate calibration model being used.

10 5) The adjunct spectral measurement can be used to correct the fluorescence spectrum and remove alterations of the fluorescence spectrum that come from known sources. For example, a diffuse reflectance spectrum can be used to correct the fluorescence spectrum for the effects of melanin or hemoglobin absorbances.

The step of combining primary and adjunct datasets may include aliasing and folding the acquired data, for example, in a Fourier Transform spectrometer, for the purpose of making robust quantitative measurements of body constituents non-invasively. Fourier-
15 transform spectroscopy ("FTS") provides a set of performance parameters completely different from dispersive spectroscopy. FTS has been widely accepted in the infrared region since 1967, and has all but totally replaced dispersive techniques in the infrared spectroscopy laboratory (H. M. Heise and R. Marbach et al., Noninvasive Blood Glucose Sensors Based on Near-Infrared Spectroscopy, 18(6) Artificial Organs 439-47,
20 1994, P. R. Griffiths, Fourier Transform Infrared Spectrometry 222(4621) Science 297-302, 1983).

Aliasing means that the information from two spectrally distinct spectral regions will be combined in one spectrum. This spectrum containing information from both the aliased and non-aliased spectral regions is then input directly into the multivariate calibration
25 model.

Alternatively, combining comprises using two or more parameters, e.g., parameters, as input variables for one or more algorithms relating to the quantitation of the analyte.

In preferred embodiment, the invention provides steps of correcting spectroscopic and non-spectroscopic parameters by at least one of the following dimensions: spectral, spatial such as skin depth and skin surface area, and temporal.

The spatial dimensions may be used to sort out the contributions from pH, electrolytes, temperature, and the like. As an example, a temperature gradient may exist between the outer surface of the skin and the tissue inside. Accordingly, the effects of temperature on the measured signal may or may not scale with the distance into the tissue normal to the tissue surface whereas contributions from electrolyte concentrations and pH may or may not be more evenly distributed. Thus, a spatial dimension is used to separate out the effects of temperature on the measured signal from those effects due to pH and electrolyte concentrations.

Temporal dimensions provide additional selectivity, which can be used to sort out contributions from electrolyte, pulsatile, pressure and temperature changes or contributions from active or passive stimulation. As a result, temporal parameters have particular value when elucidating local and/or systemic responses to varying stimuli.

Spectral dimensions represent changes in spectral form over time. Therefore, measures of spectral change as a function of time, allow the different variables to be selectively extracted, based upon a temporal dimension.

Dimensional (e.g. spectral, temporal or spatial) information obtained using these embodiments can be subjected to a combination of mathematical transformations. For example, standard statistical techniques, such as partial least squares ("PLS") analysis, or principal components regression ("PCR") analysis, can be used to correlate the absorbance of radiation at specific wavelengths to analyte structure and concentration. PLS techniques are described, for example, in Geladi et al., *Analytica Chimica Acta* 185:1-17, 1986. For a description of PCR techniques, reference may be had to Jolliffe, L. T., *Principal Component Analysis*, Springer-Verlag, New York, 1986.

These dimensions can be applied to improve analyte measurements by adjusting the original measurement before the mathematical iteration is performed to calculate the analyte concentration.

For example, the spatial dimension can be utilized to minimize the effects of the temperature gradient on fluorescence or diffuse-reflectance spectra. The first step is the derivation of the effects of a temperature gradient on fluorescence spectra and establishing a mathematical model to explain this gradient utilizing an adjunct measurement. It is possible to detect temperature using infrared spectroscopy. In this example, infrared absorption measurements could be used as a mathematical transformation on the fluorescence spectra before the calculative glucose algorithm was applied to them. The algorithm used to calculate glucose concentration would be applied after the transformation and would be made on a spectrum that was independent of temperature gradient effects (as they were accounted for by the adjunct measurement and subsequent transformation).

Temporal information can be used to correct for variations in time during the course of a measurement. For example, if the pressure applied to a probe changes over the course of a measurement, an adjunct measure of probe pressure can be used to adjust the measurements before the algorithm to calculate analyte concentration is applied to the data. In a simple situation, one can assume that pressure has a linear relationship with the measurements being taken. Thus, by recording pressure information during the time the measurements are taken, each measurement can be scaled by the constant value recorded during the course of the measurement. The transformation of the data using this adjunct measurement allows the analyte level to be calculated independent of the pressure of the device during the course of the measurement.

Spectral dimensions, as described above, refer to the change in the shape of spectra over time. Those changes that can be quantified and are not related to analyte concentration can be accounted for in the same manner as the two examples described above with an initial transformation before the application of an algorithm designed to calculate analyte concentration.

In a preferred embodiment, the invention provides a method of signal processing to improve the accessibility of chemically or physically significant information in the analytical signals. Specifically, the intensity values of signals obtained at particular wavelengths can thus be processed to reduce the effect of instrumentation noise and thus processed signals can then be subjected to multivariate analysis using known statistical techniques.

In another preferred embodiment, vibrational spectroscopy, for example, infrared or Raman, with high specificity and a relatively low signal to noise ratio is utilized to enhance calibration models built with fluorescence spectroscopy data. Specifically, this application is useful when collecting data in a common spectrum on a common frequency axis on the same spectrometer, or alternatively, collecting data with different spectrometers and later combined.

Calibration refers to the set of operations which establish, under specified conditions, the relationship between values indicated by a measuring instrument or measuring system, and the corresponding standard or known values derived from the standard. It is the process of relating data measurements for the purpose of increasing the accuracy and precision of the methodology and instrumentation in use. Statistical calibrations using chemometric methods can be used to extract specific information from a complex set of data. Such relational methods of calibration include linear regression, multiple-linear regression, partial linear regression, and principal components analysis. In other applications, calibrations can be carried out using artificial neural networks, genetic algorithms and rotated principal components analysis. The result of a calibration permits the estimation of errors of indication of the measuring instrument, measuring system, or the assignment of values so that the instrument-measuring system conforms to a known measurement output to achieve the smallest error between the instrument and the standard. In an embodiment of the invention, the step of calibrating may comprise analyzing the measurements obtained using one or more chemometric techniques, such as multivariate analysis methodologies selected from the group comprising: PLS, PCR, LDA, MLR, LR, LWR, RR, NN, stepwise LR and combinations thereof. General references for multivariate calibrations include Marten and Naes.

Instrumentation that detects information for one or more constituents in a complex chemical matrix must rely upon analysis algorithms, such as those derived using chemometrics, in order to reveal information that is specific for one or more chemical constituent. Chemometrics techniques can be used to compare unknowns with calibrated standards and databases to provide advanced forms of cluster analysis, and to extract features from an unknown patient that can be used as information in statistical and mathematical models.

Chemometrics relates to the application of mathematical, statistical and pattern recognition techniques in chemical analysis applications. See, e.g., Brown et al. (1990) Anal. Chem. 62:84-101. Chemometrics is practiced herein in the context of developing and using noninvasive diagnostic instrumentation that employs advanced signal processing and calibration techniques. Signal processing is used to improve the accessibility of physically significant information in analytical signals. Examples of signal processing techniques include Fourier transformation, first and second derivatives, and digital or adaptive filtering.

Principal components analysis ("PCA") can be performed in the application of chemometric techniques to spectroscopic measurement of chemical analytes in a complex matrix. PCA is used to reduce the dimensionality of a large number of interrelated variables while retaining the information that distinguishes one component from another. This reduction is effected using an eigenvector transformation of an original set of interrelated variables (e.g. an absorption spectrum) into a substantially smaller set of uncorrelated principal component ("PC") variables that represents most of the information in the original set. The new set of variables is ordered such that the first few retain most of the variation present in all of the original variables. See, e.g., Jolliffe, L. T., Principal Component Analysis, Springer-Verlag, New York (1986). More particularly, each PC is a linear combination of all the original measurement variables. The first is a vector in the direction of the greatest variance of the observed variables. The succeeding PCs are chosen to represent the greatest variation of the measurement data and to be orthogonal to the previously calculated PC. Therefore, the PCs are arranged in descending order of importance.

A weighting constant comprises the wavelength coefficients of partial least squares regression and/or principal components regression, or any constant obtained from any statistical calibration that can be used to calculate values (such as analyte concentration) for unknown patients. A wavelength weighting factor is an embodiment of a weighting constant which is used in the construction of an optical filter means capable of emphasizing wavelength-specific information from spectral data. The wavelength-specific information can be used to determine desired values relating to the patient undergoing analysis (e.g., analyte concentration). A wavelength weighting factor can be embodied as a particular filter density (e.g., neutral or wavelength-specific), filter thickness, or the like, such parameters having been determined using the above-described statistical calibration techniques.

In an embodiment of the invention, an apparatus for performing the above adjunct methodologies comprises a light source for exciting a target in the tissue with excitation radiation, a detection means for detecting, recording or collecting at least one spectral emission from the excited target, a determining means for determining an adjunct parameter, such as the spectral, physiological, or information parameters mentioned above, and a means for combining said spectral emission detected with said adjunct parameter to obtain the level of the at least one analyte in the tissue.

In a preferred embodiment of the invention, the apparatus comprises a display element, wherein the display element displays the level of one or more analytes and/or the adjunct parameter. The apparatus may also comprise a transmitter for transmitting data pertaining to the detected spectral emission, the adjunct parameter, the level of the at least one analyte, or any combination thereof to a centralized server, database, personal computer, or any other electronic receiver desired. The apparatus may further include an audio generator that generates one or more sounds notifying the user when analyte level(s) are determined.

One specific application for this device includes the non-invasive determination of glucose levels for the self-monitoring of glucose levels in people with diabetes. Other potential applications pertain to the identification of people with impaired glucose

metabolism such as impaired glucose tolerance, IGT, or impaired fasting glucose. People with IGT, represent a new category of pre-diabetics, and have blood glucose levels that are higher than normal but not high enough to say they have diabetes. To date, this condition has been diagnosed using an oral glucose tolerance test (OGTT). In this test, blood glucose is measured before and 2 hours after a patients drinks a glucose-containing solution, after a fast of 8 to 12 hours. About 20 million people in the U.S. have IGT, according to the National Health and Nutritional Examination Survey III. People with IGT are at higher risk for both diabetes and experiencing complications associated with chronic hyperglycemia.

10 Although the invention has been particularly shown and described with reference to several preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined in the appended claims.

EXAMPLES

15 Example 1: Acquire a fluorescence spectrum from a person; acquire a diffuse reflectance (or other adjunct spectral measurement) from the same person, and use the diffuse reflectance spectral measurement as a means by which to determine the appropriate multivariate calibration model to apply to the fluorescence measurement. Diffuse reflectance measurements contain information about skin color and skin
20 oxygenation. Various multivariate calibration models are based on fluorescence spectra that apply only to specific oxygenation levels or skin colors. The specific multivariate models have smaller errors than general models that apply to all skin colors and oxygenation levels.

Example 2: Acquire a fluorescence spectrum from a person; acquire a diffuse
25 reflectance spectral measurement (or other adjunct spectral measurement), either aliased into the fluorescence spectrum or non-aliased, then combine the two spectra (concatenate), and use them together in a single multivariate calibration model.

Example 3: Simultaneously acquire diffuse reflectance spectra and fluorescence spectra. Calculate a skin oxygen saturation value (basis set reduction) from the diffuse reflectance spectrum (using methods like, *e.g.*, Brunelle JA, Degtiarov AM, Moran RF, Race LA. Simultaneous measurement of total hemoglobin and its derivatives in blood using CO-oximeters: analytical principles; their application in selecting analytical wavelengths and reference methods; a comparison of the results of the choices made. *Scand J Clin Lab Invest Suppl.* 1996; 224:47-69). The skin oxygenation value computed from the diffuse reflectance data is then used to scale the results of the analyte concentration that had been separately computed using the skin fluorescence spectra and a multivariate calibration model. This can be done using a simple linear scaling of the form: $A_{\text{new}} = C * O_2\text{sat} * A_{\text{FI}}$, where A is the analyte value, C is a constant, $O_2\text{sat}$ is the oxygen saturation value obtained from the diffuse reflectance data, and A_{FI} is the fluorescent analyte level. It should be noted that as an alternative, oxygen saturation information may herein be supplied by a pulse oximeter as well.

It is intended that the specifications be considered exemplary only.

Claims

1. A method of determining a level of at least one analyte in a tissue comprising:
exposing the tissue with an excitation radiation from an excitation source;
detecting a spectral emission from the excited tissue;
determining an adjunct parameter selected from the group consisting of: at least one adjunct spectral emission or absorption; at least one adjunct physiological determinant; at least one adjunct informational determinant; and a combination thereof;
combining the spectral emission detected with the adjunct parameter determined, and
determining the level of said at least one analyte in the tissue.
2. The method of claim 1 wherein the excitation source is selected from group consisting of laser, visible light, ultraviolet, IR, FIR, MIR, NIR, and microwave sources and combinations thereof.
3. The method of claims 1-2 wherein exposing said tissue comprises exciting a target within said tissue.
4. The method of claim 3 wherein said target is a structural, cellular, matrix, or molecular species.
5. The method of claim 4 wherein said structural, cellular, matrix, or molecular species is selected from the group consisting of: pepsin- or collagenase-digestible collagen cross links, non-pepsin digestible collagen cross links, tryptophan, elastin, elastin cross-links, keratin, serum proteins, Glu-T proteins, NADH, NADPH, flavoproteins, FAD, melanin precursors, porphyrins, hemoglobin, glycosylated hemoglobin A1c, red blood cells, cytochromes, vitamin B complexes, carotenoid, salicylate, lactate, pyruvate, ketones, acetoacetate, beta-hydroxybutyrate, free fatty acids, succinate, fumarate, dihydroxyacetone phosphate, 3-phosphoglycerate, acetyl CoA, succinyl CoA, alpha-ketoglutarate, malate, citrate, isocitrate, bicarbonate, insulin,

triglyceride, cholesterol, phosphorus, calcium, blood urea, electrolytes, bilirubin, creatinine, albumin, lactate dehydrogenase, and combinations thereof.

6. The method of claims 1-5 wherein the level is a relative or an absolute measure.

7. The method of claims 1-6 wherein the at least one analyte is selected from the group consisting of glucose, NADH, NADPH, FAD, tryptophan, collagen, elastin, salicylate, lactate, pyruvate, ketones, acetoacetate, beta-hydroxybutyrate, free fatty acids, succinate, fumarate, DHAP, 3-phosphoglycerate, acetyl CoA, succinyl CoA, alpha-ketoglutarate, malate, citrate, isocitrate, bicarbonate, insulin, hemoglobin, glycosylated hemoglobin A1c, triglycerides, cholesterol, phosphorus, calcium, blood urea, electrolytes, bilirubin, creatinine, total protein, albumin, LDH, blood gases, cholesterol, alcohol, medications, pharmaceuticals, narcotics, poisons and combinations thereof.

8. The method of claims 1-7 wherein the tissue is selected from the group consisting of human tissue, animal tissue, forensic tissue, skin, soft tissue of the mouth, ear lobe tissue, internal body tissue, eye tissue, tissue in or around an eye, internal organ tissue, a whole organism and combinations thereof.

9. The method of claims 1-8 wherein the excitation radiation comprises a wavelength between about 295 nm to about 1100 nm.

10. The method of claims 1-9 wherein the excitation radiation comprises at a wavelength between about 320 nm to about 700 nm.

11. The method of claims 1-10 wherein the excitation radiation comprises a wavelength between about 320 nm to about 510 nm.

12. The method of claims 1-11 wherein the excitation source is selected from the group consisting of a laser, an infrared light source, a visible light source, a fluorescent light source, a microwave source, and combinations thereof.

13. The method of claims 1-12 wherein the spectral emission or absorption

comprises infrared radiation, ultraviolet, visible, diffuse reflectance, Raman or fluorescence radiation.

14. The method of claims 1-13 wherein the at least one adjunct spectral emission or absorption is detected at a wavelength between about 295 nm to about 650 nm.

15. The method of claims 1-14 wherein detecting said spectral emission or absorption comprises measuring one or more spectral characteristics of the excited target selected from the group consisting of fluorescence life-time, wavelength, intensity, relative peak ratios, spectral shapes, peak shifts, band narrowing, spectral kinetics, band broadening, scattering, polarization and combinations thereof.

16. The method of claims 1-15 wherein detection of said spectral emission or absorption is performed substantially simultaneously with determination of said adjunct parameter.

17. The method of claims 1-16 wherein the detection of said spectral emission or absorption is performed substantially sequentially with determination of said adjunct parameter.

18. The method of claims 1-17 wherein excitation of said tissue and detection of said spectral emission or absorption are performed at the same or at different locations of said tissue.

19. The method of claims 1-18 wherein the at least one adjunct spectral emission comprises infrared, near infrared, or visible absorbance.

20. The method of claims 1-19 wherein the at least one adjunct physiological determinant is determined by a passive or active procedure.

21. The method of claims 1-20 wherein at least one adjunct physiological determinant is provided by a pulse oximeter.

22. The method of claims 1-21 wherein the at least one adjunct physiological determinant is obtained from the tissue or from a patient from whom the tissue was

obtained.

23. The method of claim 22 wherein the at least one adjunct physiological determinant is a combination of two or more determinants both selected from the group consisting of: oxygenation of the tissue or the patient, tissue or patient temperature, patient blood volume, patient blood volume change, patient blood pressure, patient blood flow, blood flow of the tissue, pulsatile effects of the patient, pH of the tissue, tissue perfusion, hydration of the tissue, vasodilation of the tissue, NO or CO₂ content of the tissue, optical properties of the tissue, contact pressure affects of the tissue, stretch affects of the tissue, ultraviolet affects of the tissue, ultraviolet exposure of the tissue, and combinations thereof.

24. The method of claims 1-23 wherein the at least one adjunct informational determinant is obtained from the tissue or from a patient from whom the tissue was obtained.

25. The method of claim 24 wherein the at least one adjunct informational determinant a combination of one or more determinants selected from the group consisting of date or time of day the tissue was obtained from the patient, period of time since the patient's last meal, content of the patient's last meal, period of time since the patient's last insulin injection, patient insulin pump data, dosage of patient's last insulin injection, tissue age, tissue color, build-up of fluorescent AGEs in the tissue, sex of the patient, point in patient's menstrual cycle, tissue weight, weight or percent of patient's body fat, patient activity level, patient pulse rate or bolus, type of patient's diabetes or diabetes medications, type of patient's non-diabetes medications, patient aspirin usage, patient tobacco usage, patient glucose signal response category, and combinations thereof.

26. The method of claims 1-25 wherein combining of the spectral emission with the adjunct parameter comprises aliasing and folding.

27. The method of claims 1-26 further comprising calibrating the spectral emission against one or more other spectral emissions from said tissue using one or more

multivariate techniques.

28. The method of claim 27 wherein the one or more multivariate techniques are selected from the group consisting of PLS, PCR, LDA, RR, LR, MLR, NN, stepwise LR, LWR, and combinations thereof.

29. The method of claims 1-28 further comprising detecting a vibrational-spectroscopic emission from the excited tissue.

30. The method of claim 29 wherein the vibrational-spectroscopic emission is collected at a frequency common to the spectral emission.

31. The method of claims 1-30 further comprising adjusting the level of analyte determined by incorporating spectral information, a spatial dimension, a temporal dimension, or a combination thereof.

32. An apparatus for determining a level of at least one analyte in a tissue comprising:

a light source for exciting a tissue with excitation radiation;

a detector for detecting at least one spectral emission from the excited tissue;

a determining means for determining, recording or collecting an adjunct parameter; and

a means for combining said spectral emission detected with said adjunct parameter to obtain the level of the at least one analyte in the tissue.

33. The apparatus of claim 32 further comprising a display element, wherein said display element displays the level of the at least one analyte in the tissue.

34. The apparatus of claims 32-33 further comprising a transmitting means for transmitting data pertaining to the detected spectral emission, the adjunct parameter, the level of the at least one analyte, or a combination thereof.

35. The apparatus of claims 32-34 wherein the excitation radiation is at a

wavelength from about 295 nm to about 1100 nm.

36. The apparatus of claims 32-35 wherein the detector detects said at least one spectral emission of one or more wavelengths in the range from about 295 nm to 650 nm.

37. The apparatus of claims 32-36 wherein said analyte is selected from the group consisting of glucose, NADH, NADPH, FAD, tryptophan, collagen, elastin, salicylate, lactate, pyruvate, ketones, acetoacetate, beta-hydroxybutyrate, free fatty acids, succinate, fumarate, DHAP, 3-phosphoglycerate, acetyl CoA, succinyl CoA, alpha-ketoglutarate, malate, citrate, isocitrate, bicarbonate, insulin, hemoglobin, glycosylated hemoglobin A1c, triglycerides, cholesterol, phosphorus, calcium, blood urea, electrolytes, bilirubin, creatinine, total protein, albumin, LDH, blood gases, cholesterol, alcohol, medications, pharmaceuticals, narcotics, poisons and combinations thereof.

38. The apparatus of claims 32-37 further comprising an audio generator, wherein said audio generator generates one or more sounds when said level of at least one analyte is determined.

39. The apparatus of claims 32-38 wherein said means for combining is a processor.

40. The apparatus of claim 39 wherein the processor comprises an algorithm that combines said spectral emission and said adjunct parameter.

41. The apparatus of claims 32-40 further comprising a memory.

42. The apparatus of claims 32-41 which provides real time information on said level of the at least one analyte.

43. The apparatus of claims 32-42 which provides trending information on said level of the at least one analyte.

44. The apparatus of claims 32-43 wherein the adjunct parameter is provided by a pulse oximeter.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
6 February 2003 (06.02.2003)

PCT

(10) International Publication Number
WO 2003/010510 A3

(51) International Patent Classification:
15/06

G01N 33/53,

(74) Agents: ELRIFI, Ivor, R. et al.; Mintz, Levin, Cohn,
Ferris, Glovsky and Popeo, P.C., One Financial Center,
Boston, MA 02111 (US).

(21) International Application Number:

PCT/US2002/023348

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,
ZW.

(22) International Filing Date: 24 July 2002 (24.07.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/307,389

25 July 2001 (25.07.2001) US

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK,
TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

(71) Applicant (*for all designated States except US*): AR-
GOSE, INC. [US/US]; 230 Second Avenue, Waltham,
MA 02451 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): MESSER-
SCHMIDT, Robert, G. [US/US]; 100 Broadway
Boulevard, NE, #205, Albuquerque, NM 87102 (US).
MANSFIELD, James [US/US]; 22-25 Peterborough
Street, Apt. 22, Boston, MA 02215 (US). BRAND, Derek
[US/US]; 11 Bentley Street, Brighton, MA 02135 (US).
FREEMAN, Jenny, E. [US/US]; 405 Concord Road,
Weston, MA 02493 (US). HOPMEIER, Michael, J.
[US/US]; 425 E. Hollywood Blvd., Suite A, Mary Ester,
FL 32580 (US).

Published:

— with international search report

(88) Date of publication of the international search report:
19 February 2004

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: ADJUNCT QUANTITATIVE SYSTEM AND METHOD FOR NON-INVASIVE MEASUREMENT OF IN VIVO AN-
ALYTES

(57) Abstract: The invention is directed to a method and apparatus for detecting and measuring the concentration of an analyte in
a tissue and a patient. Particularly, the invention is directed to methods wherein a spectroscopic measurement is combined with an
adjunct spectroscopic or non-spectroscopic measurement to provide a more accurate measure of the analyte. The non-spectroscopic
measurements include adjunct spectral measurements, adjunct physiological measurements of the patient, the tissue or the patient
from whom the tissue was obtained, or adjunct informational determinants. Preferably these methods can be used for determining
the level of glucose in a patient and for enhancing a calibration system.

WO 2003/010510 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/23348

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01N 33/53; G01N 15/06

US CL : 435/7.1; 422/68.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1; 422/68.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,985,214 A (STYLLI et al.) 16 November 1999(16.11. 1999), see entire document, especially columns 23-32.	1-5 and 32-34
X	US 5,665,962 A (KIMURA et al.) 09 September 1997 (09.09.1997), see whole document, especially columns 5-11.	32-34

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

28 September 2003 (28.09.2003)

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Facsimile No. (703) 305-3230

Date of mailing of the international search report

03 DEC 2003

Authorized officer

Frank Lu

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/23348

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claim Nos.: 6-31 and 35-44
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/US02/23348

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

- Group I, claims 1-5, drawn to a method of determining a level of at least one analyte in a tissue.
- Group II, claims 32-34, drawn to an apparatus for determining a level of at least one analyte in a tissue.

and it considers that the International Application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below:

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I and II do not relate to a single inventive concept because they lack the same or corresponding special technical features since an apparatus is known in the art. For example, since claim 32 only requires a light source that can excite a tissue with excitation radiation, a detector for detecting at least one spectral emission from the excited tissue, a determining means that can determine, record or collect an adjunct parameter and a means that can combine said spectral emission detected with said adjunct parameter to obtain the level of the at least one analyte in the tissue while Kimura's patent teaches all components recited in claim 32 (US Patent No. 5,665,962, published on September 9, 1997), the technical feature of Group II is not special.

Continuation of B. FIELDS SEARCHED Item 3:

STN AND WEST

Search terms: exciation, radiation, spectral, emission, adjunct, parameter, determine or determination, determining means, transmitting means, and parameter

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☒ **BLACK BORDERS**

☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**

☐ **FADED TEXT OR DRAWING**

☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**

☐ **SKEWED/SLANTED IMAGES**

☒ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**

☐ **GRAY SCALE DOCUMENTS**

☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**

☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**

☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.